Iron Transport in Pea Plants[1, 2]

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Iron has long been recognized as an essential element for plant growth, but there has been little effort to determine how iron moves in normal, green plants. Attention has focused on chlorosis, which has frequently been ascribed to iron immobilization or inactivation rather than to iron deficiency (5, 6, 30, 31, 32). However, recent evidence shows a clear relationship between total iron content and chlorophyll content (2, 20, 21, 29). Previous evidence to the contrary can often be explained as a failure to wash leaves prior to analysis (20), or a failure to maintain a constant supply of iron (21). Viewing iron chlorosis as an iron deficiency emphasizes the need to study iron uptake and transport rather than iron inactivation.

Ion uptake from a nutrient solution into the roots and to the shoots of a plant has been described as an active process, dependent upon the metabolic activity of the root cells (4, 7, 34), and as a passive process dependent upon mass flow in the transpiration stream (11, 17, 18, 19, 22). The opposing viewpoints were recently reviewed by Russell and Barber (33). Many of the relations between transpiration and ion transport were elucidated by Broyer and Hoagland (7). The important feature of their conclusion is that it postulates an active secretion into the xylem—possibly against a concentration gradient. On the other hand, Hylmo's (17) extensive experiments showed a close correlation between transpiration and salt uptake, and led him to believe that such metabolic processes are of little importance, especially for polyvalent ions. The evidence that Ca^{++} is very slowly accumulated by excised barley roots (26) appeared to support this contention and suggested the possibility that iron moves passively across the root in the transpiration stream.

Schmid and Gerloff (35) have shown that a naturally occurring iron chelate could account for iron movement in the xylem at pH's which would otherwise cause it to precipitate. But how does iron, at these same pH's, get into the plant in the first place? The unique problems which are posed by iron's insolubility at biological pH's have led many investigators to conclude that plants growing in the soil may take up iron from insoluble particles (9, 12, 15). A similar phenomenon may occur in solution-grown plants, though in this case it has generally been implied that iron is taken up from a soluble phase. For example, Glauser and Jenny (12), who are strong proponents of a contact mechanism for nutrient uptake from soils, stated that their percolating nutrient solution at pH 6.1 contained enough dissolved iron to satisfy the needs of their plants. Rather than dissolved iron, it is possible they were dealing with colloidal iron which would also have passed through their percolating system. In other cases, the passage of nutrient solution-iron through filter paper (28, 38) has encouraged the belief that more iron remains in solution than calculations based on the solubility product of $Fe(OH)_3$ predict. However, there is no evidence to substantiate this belief.

This study was undertaken to answer two of the basic questions concerning iron transport in green plants: A, Is the passage of iron from a nutrient solution to the shoots a passive process, or is it dependent upon the metabolic activity of the root? B, Do plants growing in inorganic nutrient solutions take up iron from insoluble iron particles?

Materials & Methods

Pea plants (*Pisum sativum*, L., var. Alaska) were used in all of the experiments. The pea seeds were germinated in the dark at 25 C by soaking for 24 hours in aerated, glass-distilled water. The seeds were then placed on a screen over aerated 1/10 strength Hoagland solution. The plants remained in the dark at 25 C for 2 more days. They were then removed from the dark and planted in beakers by threading their roots through polyethylene tops. In the first experiment described below, 40 plants were placed in each of sixteen 150-ml beakers. In subsequent experiments, 16 plants were placed in each of twelve 50-ml beakers. The beakers were wrapped in aluminum foil, filled with half strength Hoagland solution and placed in a circle under a 750 w incandescent bulb. The plants continued to grow in half strength Hoagland solution until they

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were 9 days old. All nutrient solutions were purified by the method of Stout and Arnon (37). Constant aeration was provided, and the solutions were changed daily. The 750 w bulb was turned on and off to provide a 12 hour light-dark cycle. Ambient temperature varied daily from 21 C to 28 C.

For an experiment, the groups of 16 (or 40) nine-day old plants were transferred, in their polyethylene beaker-tops, to a 30 second distilled water rinse and then to the experimental solution. Besides Fe^{59} or in some cases Rb^{86}, the experimental solutions contained only $CaSO_4$ (1 meq/l) and KCl. The amount of K^+ varied from 1 to 2 meq/liter, depending upon how much KOH was required to neutralize the HCl in which the radioactive materials were supplied.

Transpiration was calculated from the total weight loss of a beaker and its contents. The weight loss from identical beakers containing no plants provided a measure of loss by evaporation. Transpiration is expressed as grams of H_2O lost per beaker. It amounted to about one kilogram of H_2O in three hours per kilogram fresh weight of the shoots. In some cases transpiration was reduced by placing the plants in a dark, humid atmosphere. A large number of preliminary experiments indicated that this procedure was a reliable and reproducible means of effecting a tenfold decrease in the transpiration rate.

The plant tops were harvested by cutting the stem just above the second trifid bract. Total iron was determined by a modified bathophenanthroline method (36). For Fe^{59} or Rb^{86} determinations, the fresh shoots or aliquots of the digested shoots were placed in counting vials and counted in a Tracerlab well-type scintillation counter. In most cases, results of these determinations are given as relative activity, a figure representing the average counts/minute in 1 kg fresh weight of the tops divided by the counts/minute in 1 ml of the nutrient solution. This expression allows rough comparison between the tables to follow, though exact quantitative comparison between two different experiments should not be made.

In some experiments, decorticated plants were used. These plants were prepared 24 hours before the start of an experiment by hand stripping the cortical tissue off the central vascular column of the tap root. Decortication severed the lateral roots and tore the radial walls of the endodermal cells, leaving portions of their suberized walls hanging from the decorticated stele. Thus, the vascular column of what remained of the root was not covered by an intact endodermal layer, and the xylem elements of what had been lateral roots opened directly into the nutrient solution. These plants could remain alive and grow slowly for at least two weeks after decortication.

Results

A preliminary experiment was performed to determine if isotopic dilution in the roots would impugn the reliability of conclusions based on radioactivity measurements alone. The experimental treatments and results are indicated in table I. With intact seedlings much of the iron reaching the shoots came from the cotyledons; removing them markedly reduced the total iron transported but barely affected the movement of labelled iron from the solution to the shoots. With the cotyledons removed, the total iron reaching the shoots coincided within experimental error with the transport of an equivalent amount of labelled iron. The results suggest that isotopic dilution in the roots was insufficient to bias conclusions based on activity measurements.

Table I also shows that prior growth in iron-free media decreased the subsequent movement of solution-iron to the shoots during the experimental treatment. Typical of these observations are the results of another experiment, recorded in figure 1. Groups of

Table I

Total & Labelled Fe Transport After Different Iron Pretreatments

Iron pretreatment*	Experimental treatment**	Increase of Fe in shoots (μg/plant) Total***	Labelled†
+Fe	Intact plants	0.36±0.04	0.077±0.005
+Fe	Cotyledons removed	0.08±0.02	0.061±0.005
−Fe	Intact plants	0.12±0.02	0.011±0.001

* All plants grown at pH 5.5 in half strength Hoagland solution; +Fe: received 0.5 ppm Fe as $FeCl_3$ for 48 hour pretreatment; −Fe: received no iron during pretreatment.

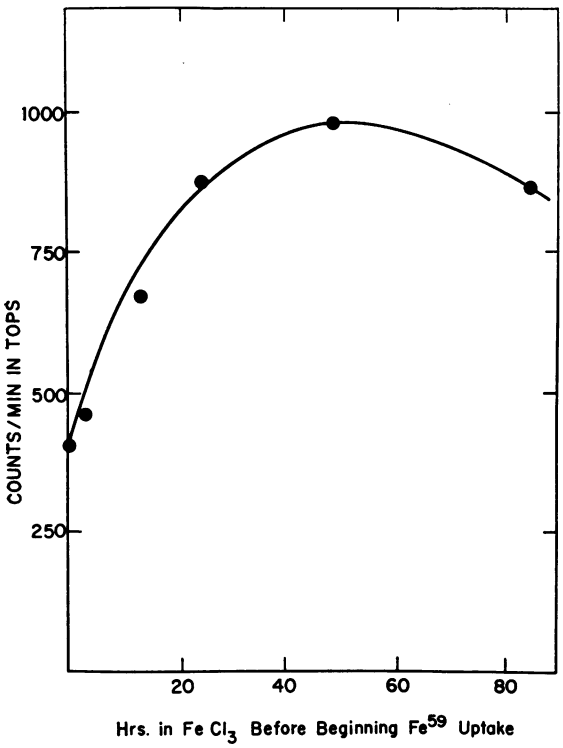
** All treatments at 0.5 ppm labelled Fe (8 μc/mg Fe) added as FeEDTA; initial pH of all solutions 5.5; uptake period 3 hours. Where indicated, cotyledons were removed just prior to the uptake period.

*** Determined chemically. Each figure represents the difference between the average of nine replicate samples (9 plants per sample) taken at the start and at the end of the uptake period. The standard deviation is shown.

† Each figure represents the average of nine replicate samples (9 plants per sample) taken at the end of the uptake period. The standard deviation is shown.

plants which had grown in nutrient solutions free of iron were pretreated for varying time periods in nutrient solutions containing 0.5 ppm Fe added as $FeCl_3$. Then all the plants were placed for 4 hours in a solution containing 0.5 ppm Fe as $FeCl_3$ labelled with Fe^{59}. As figure 1 shows, the least Fe^{59} moved to the tops of plants receiving no Fe pretreatment. There were no visually observable differences between plants pretreated in $FeCl_3$ and those which remained in iron-free solutions. None of the plants showed signs of chlorosis and all had made equally rapid growth.

The results of figure 1 may be explained by assuming that rapid, active accumulation of iron by previously iron-starved root cells competes with the passive flow of iron to the shoots. If this model is correct, it follows that for short time intervals:



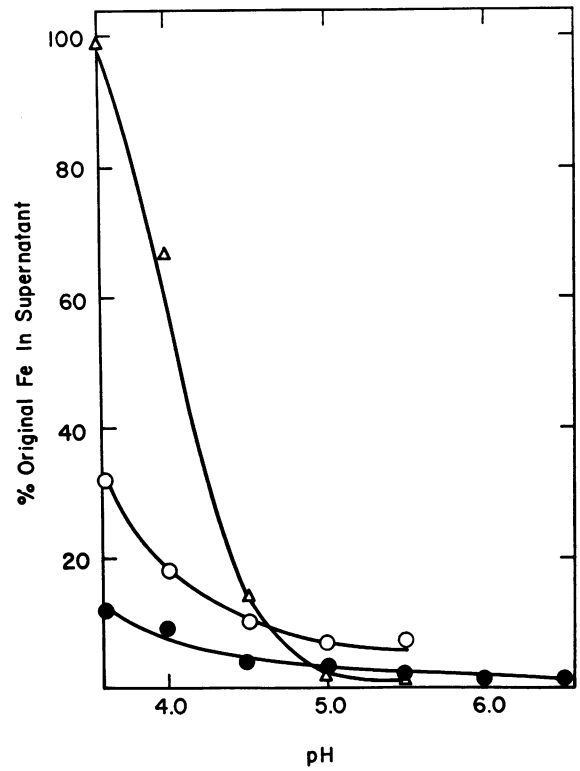


Fig. 1 (*left*). The effect of iron pretreatment on subsequent iron transport. Plants grown at pH 5.0 in the absence of iron were pretreated for varying time periods in half-strength Hoagland solutions, pH 5.0, containing 0.5 ppm Fe as $FeCl_3$. After pretreatment, the plants were placed in experimental solutions labelled with Fe^{59} (10 μc/mg Fe) containing 0.5 ppm Fe added as $FeCl_3$, initial pH 5.0. Uptake period 4 hours.

Fig. 2 (*right*). Iron concentration as a function of pH in supernatant of centrifuged solutions to which 0.5 ppm Fe was added as $FeCl_3$ labelled with Fe^{59}. The curves show Fe added to: ●——● Half-strength Hoagland solution centrifuged 3 hours, ○——○ Half-strength Hoagland solution centrifuged 30 minutes, and △——△ distilled water centrifuged 30 minutes. Continued precipitation during successively longer centrifugation periods should not be interpreted as demonstrating slow hydrolysis; solutions stored in the laboratory for equivalent time periods were precipitated at the same rate when later placed in the centrifuge.

I. Slowing the transpiration rate should decrease iron translocation to the shoots, and

II. Preventing the active accumulation of iron in previously iron-starved root cells should increase iron translocation to the shoots. Transpiration was decreased by placing some of the plants in a dark, humid atmosphere. Active accumulation of iron by the root cells was modified in two ways: addition of 2,4-dinitrophenol (DNP) to one group of plants and complete removal of the cortex in another group of plants. The results are given in table II. Reducing transpiration substantially decreased iron movement to the tops. But preventing active accumulation in the root cells did not increase translocation to the shoots. Neither the addition of DNP nor the removal of the cortex increased iron movement in rapidly transpiring plants — regardless of prior growth conditions. Less labelled iron was translocated in DNP-treated plants than in the controls. Virtually no Fe^{59} was translocated in decorticated plants. This was particularly surprising since movement of Rb^{86}, used here for comparison, was not so affected by decortication.

The striking effect of decortication on iron movement suggested a careful re-examination of the nutrient solutions used. Calculations based on the dissociation constant of $Fe(OH)_3$ show that less than 10^{-6} ppm Fe^{+++} remains in solution at pH 5.5. Since such calculations assume, among other things, equilibrium conditions, experiments were conducted to verify their applicability. Solutions containing 0.5 ppm Fe added as $FeCl_3$ were freshly prepared and adjusted to the desired pH with KOH or HCl. Tracer amounts of Fe^{59} were included. The solutions were centrifuged at $2 \times 10^4 \times g$ at 20 C for varying time periods. The supernatant was sampled and the percentage original iron remaining was calculated. Typical results given in figure 2 indicate that hydrolysis occurred rapidly and that very little iron remained in solution at pH 5.5. Furthermore, examination of our dilute, freshly prepared $FeCl_3$ "solutions" in the electron micro-

Table II

Effect of DNP, Decortication, & Transpiration on Fe Transport

Iron pretreatment*	Experimental treatment**	Transpiration		Relative activity in shoots***
		Conditions	ml/beaker	
+Fe	$Fe^{59}Cl_3$	High	4.7	147
−Fe	$Fe^{59}Cl_3$	High	4.2	38
+Fe	$Fe^{59}Cl_3$	Low	~0.5	38
+Fe	$Fe^{59}Cl_3$ + DNP	High	4.7	91
−Fe	$Fe^{59}Cl_3$ + DNP	High	4.2	8
+Fe	$Fe^{59}Cl_3$, decorticated	High	4.5	~0
−Fe	$Fe^{59}Cl_3$, decorticated	High	4.8	~0
+Fe	$Rb^{86}Cl$	High	4.6	521
+Fe	$Rb^{86}Cl$, decorticated	High	4.2	545

* All plants grown at pH 5.5 in half strength Hoagland solution; +Fe: received 0.5 ppm Fe as $FeCl_3$ for 48 hour pretreatment; −Fe: received no iron during pretreatment.

** Labelled Fe (20 μc/mg Fe) added at 0.5 ppm as $FeCl_3$; labelled Rb (about 0.1 μc/mg Rb- at 1 meq/liter; DNP, where indicated, added at 5×10^{-5} M. Initial pH of all solutions 5.5; uptake period 3 hours.

*** Relative activity = counts/minute in 1 kg fresh weight of shoots divided by counts/minute in 1 ml nutrient solution. Each figure is the average of three replicate samples, three plants per sample.

scope revealed particles similar to Grunes & Jenny's (14) $Fe(OH)_3$ prepared by heating and long ripening of concentrated $FeCl_3$ solutions.

These observations showed that lack of iron transport in decorticated plants could be related to the absence of any significant amounts of dissolved iron in the nutrient solution used. To test this assumption plants were placed in solutions of iron solubilized by chelation with ethylenediamine tetraacetate (EDTA) (table III). In this case, iron readily moved to the tops of decorticated plants. In normal plants, DNP depressed labelled iron movement from FeEDTA solutions, as it had from $FeCl_3$ "solutions." In contrast, DNP had no effect on iron movement in decorticated plants in FeEDTA solutions. Though low transpiration decreased iron translocation in normal plants, this effect was not as pronounced as in decorticated plants. Rb^{86} movement, included in table III for comparative purposes, showed a similar relation between normal and decorticated plants.

The striking effect of chelation on iron movement into decorticated plants suggested that decorticated

Table III

Effect of DNP, Decortication, & Transpiration on Fe & Rb Transport*

Experimental treatment**	Transpiration		Relative activity in shoots***
	Conditions	ml/beaker	
Fe^{59}EDTA, decorticated plants	High	...	773
Fe^{59}EDTA, decorticated plants	Low	...	72
Fe^{59}EDTA, decorticated plants + DNP	High	...	808
Fe^{59}EDTA, normal plants	High	...	286
Fe^{59}EDTA, normal plants	Low	...	73
Fe^{59}EDTA, normal plants + DNP	High	...	73
Fe^{59}EDTA, normal plants + DNP	Low	...	12
$Rb^{86}Cl$, decorticated plants	High	4.0	406
$Rb^{86}Cl$, decorticated plants	Low	~0.5	45
$Rb^{86}Cl$, normal plants	High	4.3	472
$Rb^{86}Cl$, normal plants	Low	~0.5	146
$Rb^{86}Cl$, normal plants + DNP	High	4.2	155
$Rb^{86}Cl$, normal plants + DNP	Low	0.6	61

* All plants grown at pH 5.5 in half strength Hoagland solution in the absence of Fe.

** Labelled Fe (3 μc/mg Fe) added at 0.5 ppm as FeEDTA; labelled Rb (about 0.1 μc/mg Rb) at 1 meq/liter. Initial pH of all solutions 5.5; uptake period 3 hours.

*** Relative activity = counts/minute in 1 kg fresh weight of shoots divided by counts/minute in 1 ml nutrient solution. Each figure is the average of three replicate samples, three plants per sample.

plants could be used to test the hypothesis that roots excrete organic materials which complex iron in the surrounding medium (16). Decorticated plants were placed together with normal plants in one beaker. Though the roots of the normal plants were in the same solution as, and intertwined with, the vascular tissues of the decorticated plants, no labelled iron moved to the tops of the latter. In another experiment, the excised cortex from one group of plants was placed in a beaker containing another group of decorticated plants. Again, no solution iron moved to the tops of decorticated plants.

In all of the experiments described above, the concentration of iron added to the nutrient solution was kept constant. In the next experiment, plants grown in the absence of iron were placed in nutrient solutions which contained varying amounts of $FeCl_3$, as indicated in table IV. In this case the activity determinations were used to calculate the amount of iron reaching the shoots from the roots. Table IV indicates that decreasing the concentration of iron from 5 ppm to 0.5 ppm did not result in concomitant decreases in the amount of iron reaching the shoots. Similar data were obtained using plants which had grown in the presence of iron.

Table IV

Effect of Fe Concentration on Fe Transport*

Experimental treatment**	Increase of Fe in shoots*** (μg/kg fr wt)
5.0 ppm Fe	18
1.0 ppm Fe	21
0.5 ppm Fe	19

* All plants grown at pH 5.5 in half strength Hoagland solution in the absence of Fe.

** Labelled Fe (2, 10, or 20 μc/mg Fe, respectively) added as $FeCl_3$ at the concentration indicated. Initial pH of all solutions 5.5; uptake period 3 hours.

*** Calculated from activity in shoots and specific activity of solutions. The calculation reflects only the iron which is coming from the solution. Each figure is the average of three replicate samples, three plants per sample.

Discussion

Hylmo (17), Epstein (11), and Kramer (22) have described ion uptake and translocation in terms of two parallel processes: active accumulation by the roots and passive movement of ions through free space to the xylem and thence to the shoots in the transpiration stream. If (fig 1) active accumulation of iron by previously iron-starved root parenchyma is assumed to compete with passive flow of iron to the xylem, inhibiting this accumulation should have increased iron translocation to the shoots. It did not (table II). Less labelled iron was translocated in DNP-treated plants than in the controls—regardless of whether or not the plants had previously received iron. In normal plants, DNP also inhibited iron transport from FeEDTA solutions (table III). In all normal plants, iron transport was reduced in the presence of DNP; transpiration was not. Similar results were observed by Butler (8) and Brouwer (4) in their studies of chloride uptake in wheat and broad bean plants. However, Hylmo (18) claimed that DNP eliminated only the active component ("active bleeding") of transport and that the salt uptake in the presence of DNP was still proportional to water uptake. He further suggested that transport of the active component also is partly dependent upon water movement. But the data of tables II and III show that DNP reduced the transport of labelled iron in normal plants by 40 to 80 %, depending upon the particular treatment and history of the plant. Though it is likely the conditions under which the DNP was used, i.e. pH, concentration, etc., caused only partial inhibition, at least a substantial part (more than 75 % in most cases) of the iron transport was related to the cellular metabolism of the roots and could be inhibited by DNP. Similar considerations and conclusions apply to the transport of Rb from RbCl solutions (table III).

Though both table II and table III show transpiration affected the rate of Fe and Rb transport, comparison of normal and decorticated plants (table III) suggests transpiration exerted only an indirect effect in normal plants. Decorticated plants were an excellent model for study of passive transport. Iron movement in decorticated plants seemed to be completely passive since, in such plants, DNP had no effect on FeEDTA translocation. Movement of both Fe (from FeEDTA solutions) and Rb to the tops of decorticated plants was decreased tenfold by conditions causing a tenfold decrease in transpiration; in normal plants similar changes in transpiration conditions caused smaller decreases in Fe or Rb translocation.

Neither the DNP effects nor the transpiration relations which were observed in this study are adequately explained by the passive flow hypothesis. On the other hand, all of the data are consistent with the view that salt transfer across the root to the stele is an active process. Figure 1 may be explained by assuming that sufficient amounts of iron had to be accumulated in root parenchyma before transfer to the xylem occurred, an assumption which was substantiated by radioautographic evidence to be presented in a subsequent paper (3).

Broyer and Hoagland's (7) hypothesis that transpiration promotes salt excretion into the xylem predicts the observations in table III. Decorticated plants responded as a wick to changes in transpiration and were insensitive to DNP. In normal plants active transfer of salts to the xylem partly counteracted low transpiration conditions by increasing concentrations of Fe (or Rb) in the transpiration stream. Normal plants were sensitive to DNP. Radioautographs (3) showed that DNP inhibited iron absorption in the roots; tables II and III show that DNP inhibited iron transport to the shoots, suggesting that the two processes of iron absorption and iron transport are mutually interdependent.

Iron from $FeCl_3$ "solutions" at pH 5.5 was not translocated to the shoots of decorticated plants (table II). Since chelated iron and Rb were readily translocated in decorticated plants, the easiest explanation for lack of $FeCl_3$ transport is that an insignificant amount of iron was in solution at pH 5.5. Centrifugation experiments bear out this conclusion and suggest that plants take up iron from insoluble particles. At pH 5.5 less than 2% of the original iron remained in the supernatant of centrifuged $FeCl_3$ preparations (fig 2). Though concentrated solutions of $FeCl_3$ are known to hydrolyze slowly (23, 24), the experiments reported here bear out the conclusions of Lamb and Jacques (23, 24) that iron hydrolyzes rapidly when in dilute concentrations.

Concentration experiments (table III) support the hypothesis that iron is taken up from solid particles. Despite tenfold changes (from 5–0.5 ppm Fe) in the nutrient, no change in the amount of iron transported to the shoots was observed. Other investigators (1, 31) also noted that the concentration of inorganic iron supply in their nutrient did not have a proportional effect on Fe uptake. If the iron were in solution, one might anticipate changes in concentration to cause changes in the rate of iron transport. If, on the other hand, uptake of iron from $FeCl_3$ occurred from iron particles which completely covered the surface of the root at 0.5 ppm, further addition of $FeCl_3$ would not be expected to cause any increase in iron uptake.

Several hypotheses to account for iron uptake have been advanced. Hutner et al. (16) suggested that roots excrete metal-solubilizing substances. This suggestion has received little experimental support (28), and our results using decorticated plants as a bioassay for such compounds also failed to detect their presence. Jenny and his co-workers (10, 12, 13) proposed that plants take up iron by direct interaction of the root surface with iron particles in the soil. According to their scheme, carboxyl groups associated with cell wall material at the root surface may acquire iron by a contact decomposition process involving exchange of hydrogen ions for iron. If, however, colloidal particles of iron were able to diffuse through the loose-textured, hydrated primary walls of the root epidermis (27), direct interaction and complex formation between the plasmalema constituents and the colloidal iron particles could occur. On the other hand, entry of large protein molecules into barley root cells, presumably by pinocytosis, has been observed by McLaren et al. (25), and a similar mechanism might allow direct entry of iron particles into the cytoplasm.

Summary

Iron transport in intact pea plants was studied. The rate of iron movement from a nutrient solution to the shoots was measured under various conditions of transpiration, metabolic inhibition, and iron concentration. Chemical and radiochemical determinations were used to measure the rate of iron transport.

In short term experiments, less solution iron was transferred to the shoots of plants grown in the absence of iron than to the shoots of plants grown in the presence of iron. These differences were explained by assuming the root cells of the iron-starved plants absorbed iron to meet their own requirements before transferring it to the transpiration stream.

Decorticated plants served as a model system in which passive flow in the transpiration stream could be observed. Comparison of decorticated and normal plants indicated that iron transport from the nutrient solution to the shoots was dependent upon the metabolic activity of the root cells.

Normal, intact plants were able to take up iron from colloidal particles on the root surface.

Literature Cited

1. AGARWALA, S. C. & C. P. SHARMA. 1961. The relation of iron supply to the tissue concentration of iron, chlorophyll, & catalase in barley plants grown in sand culture. Physiol. Plantarum 14: 275–283.
2. BENNETT, J. P. 1945. Iron in leaves. Soil Science 60: 91–105.
3. BRANTON, D. & L. JACOBSON. 1962. Iron localization in pea plants. Plant Physiol. 37: 546–551.
4. BROUWER, R. 1956. Investigation into the occurrence of active & passive components in the ion uptake by *Vicia faba*. Acta Botan. Neerl. 5: 287–314.
5. BROWN, J. C. 1956. Iron chlorosis. Ann. Rev. Plant Physiol. 7: 171–190.
6. BROWN, J. C., L. O. TIFFIN, R. S. HOLMES, A. W. SPECHT, & J. W. RESNICKY. 1959. Internal inactivation of iron in soybeans as affected by root growth medium. Soil Sci. 87: 89–94.
7. BROYER, T. C. & D. R. HOAGLAND. 1943. Metabolic activities of roots & their bearing on the relation of upward movement of salts & water in plants. Am. J. Botany 30: 261–273.
8. BUTLER, G. W. 1953. Ion uptake by young wheat plants. II. The "apparent free space" of wheat roots. Physiol. Plantarum 6: 617–635.
9. CHAPMAN, H. D. & G. F. LIEBIG. 1939. Absorption of iron from finely ground magnetite by citrus seedlings. Soil Sci. 48: 309–315.
10. CHARLEY, J. L. & H. JENNY. 1961. Two-phase studies on availability of iron in calcareous soils. IV. Decomposition of iron oxide by roots, & Fe-diffusion in roots. Agrochimica 5: 99–107.
11. EPSTEIN, E. 1960. Spaces, barriers, & ion carriers: Ion absorption by plants. Am. J. Botany 47: 393–399.
12. GLAUSER, R. & H. JENNY. 1960. Two-phase studies on availability of iron in calcareous soils. I. Experiments with alfalfa plants. Agrochimica 4: 263–278.
13. GLAUSER, R. & H. JENNY. 1961. Two-phase studies on availability of iron in calcareous soils. III. Contact & exchange diffusion in ionic membranes. Agrochimica 5: 1–9.
14. GRUNES, D. L. & H. JENNY. 1960. Two-phase studies on availability of iron in calcareous soils. II. Decomposition of colloidal iron hydroxide by ion exchangers. Agrochimica 4: 279–287.

15. GUEST, P. L. 1944. Root-contact phenomena in relation to iron nutrition & growth of citrus. Proc. Am. Soc. Hort. Sci. 44: 43–48.
16. HUTNER, S. H., L. PROVASOLI, A. SCHATZ, & C. P. HASKINS. 1950. Some approaches to the study of the role of metals in the metabolism of microorganisms. Proc. Am. Philosophical Soc. 94: 152–170.
17. HYLMO, B. 1953. Transpiration & ion absorption. Physiol. Plantarum 6: 333–405.
18. HYLMO, B. 1955. Passive components in the ion absorption of the plant. I. The zonal ion & water absorption in Brower's experiments. Physiol. Plantarum 8: 433–449.
19. HYLMO, B. 1958. Passive components in the ion absorption of the plant. II. The zonal water flow. ion passage, & pore size in roots of *Vicia faba*. Physiol. Plantarum 11: 382–400.
20. JACOBSON, L. 1945. Iron in the leaves & chloroplasts of some plants in relation to their chlorophyll content. Plant Physiol. 20: 233–245.
21. JACOBSON, L. & J. J. OERTLI. 1956. The relationship between iron & chlorophyll content in chlorotic sunflower leaves. Plant Physiol. 31: 199–204.
22. KRAMER, P. J. 1957. Outer space in plants. Science 125: 633–635.
23. LAMB, A. B. & A. G. JACQUES. 1938. The slow hydrolysis of ferric chloride in dilute solution. I. The change in conductance, color, & chloride ion concentration. J. Am. Chem. Soc. 60: 967–981.
24. LAMB, A. B. & A. G. JACQUES. 1938. The slow hydrolysis of ferric chloride in dilute solution. II. The change in hydrogen ion concentration. J. Am. Chem. Soc. 60: 1215–1228.
25. MCLAREN, A. D., W. A. JENSEN, & L. JACOBSON. 1960. Absorption of enzymes & other proteins by barley roots. Plant Physiol. 35: 549–556.
26. MOORE, D. P., L. JACOBSON, & R. OVERSTREET. 1961. Uptake of calcium by excised barley roots. Plant Physiol. 36: 53–57.
27. MUHLETHALER, K. 1961. Plant cell walls. In: The Cell, Vol. II. J. Brachet & A. E. Mirsky, eds. Pp. 85–134. Academic Press, New York.
28. OERTLI, J. J. 1956. Studies in the uptake of iron by higher plants. Ph.D. Thesis, University of California.
29. OERTLI, J. J. & L. JACOBSON. 1960. Some quantitative considerations in iron nutrition of higher plants. Plant Physiol. 35: 683–688.
30. OLSEN, C. 1935. Iron absorption & chlorosis in green plants. Compt. rend. trav. lab. Carlsberg. Ser. chim. 21: 15–52.
31. REDISKE, J. H. & O. BIDDULPH. 1953. The absorption & translocation of iron. Plant Physiol. 28: 576–593.
32. ROGERS, C. H. & J. W. SHIVE. 1932. Factors affecting the distribution of iron in plants. Plant Physiol. 7: 227–252.
33. RUSSELL, R. S. & D. A. BARBER. 1960. The relationship between salt uptake & the absorption of water by intact plants. Ann. Rev. Plant Physiol. 11: 127–140.
34. RUSSELL, R. S. & V. M. SHORROCKS. 1959. The relationship between transpiration & absorption of inorganic ions by intact plants. J. Exptl. Botany 10: 301–316.
35. SCHMID, W. E. & G. C. GERLOFF. 1961. A naturally occurring chelate of iron in xylem exudate. Plant Physiol. 36: 226–231.
36. SMITH, G. F., W. H. MCCURDY, JR. & H. DIEHL. 1952. The colorimetric determination of iron in raw & treated municipal water supplies by use of 4: 7-diphenyl-1: 10-phenanthroline. Analyst 77: 418–422.
37. STOUT, P. R. & D. I. ARNON. 1939. Experimental methods for the study of the role of copper, manganese, & zinc in the nutrition of higher plants. Am. J. Botany 26: 144–149.
38. WALLIHAN, E. F. 1961. Effect of sodium bicarbonate on iron absorption by orange seedlings. Plant Physiol. 36: 52–53.